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N deposition and elevated CO₂ on methane emissions: Differential responses of indirect effects compared to direct effects through litter chemistry feedbacks

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[1] Increases in atmospheric CO₂ concentration and N deposition are expected to affect methane (CH₄) production in soils and emission to the atmosphere, directly through increased plant litter production and indirectly through changes in substrate quality. We examined how CH₄ emission responded to changes in litter quality under increased N and CO₂, beyond differences in CH₄ resulting from changes in litter production. We used senesced leaves from ¹³C-labeled plants of *Molinia caerulea* grown at elevated and ambient CO₂ and affected by N fertilization to carry out two experiments: a laboratory litter incubation and a pot experiment. N fertilization increased N and decreased C concentrations in litter whereas elevated CO₂ decreased litter quality as reflected in litter C and N concentrations and in the composition of lignin and saturated fatty acids within the litter. In contrast to our expectations, CH₄ production in the laboratory incubation decreased when using litter from N-fertilized plants as substrate, whereas litter from elevated CO₂ had no effect, compared to controls without N and at ambient CO₂. Owing to high within-treatment variability in CH₄ emissions, none of the treatment effects were reflected in the pot experiment. C mineralization rates were not affected by any of the treatments. The decrease in CH₄ emissions due to indirect effects of N availability through litter quality changes (described here for the first time) contrast direct effects of N fertilization on CH₄ production. The complex interaction of direct effects with indirect effects of increased N on litter quality may potentially result in a net decrease in CH₄ emissions from wetlands in the long term.

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1. Introduction

[2] Methane (CH₄) is the second most important trace gas after CO₂. CH₄ concentrations have more than doubled since preindustrial times (1750) and have risen by 1% per year during the last century. Although the rate of increase has slowed to nearly zero during the last decade [Solomon *et al.*, 2007], the most recent measurements show renewed growth from the end 2006 [Rigby *et al.*, 2008].

[3] Atmospheric concentrations of CH₄ are partially determined by soil carbon cycling, given that CH₄, like CO₂, is an end product of soil organic matter decomposition and carbon (C) mineralization [Tsutsuki and Ponnamperna, 1987]. The principal factors controlling methane produc-

tion from soil organic matter decomposition have been thoroughly quantified over the last two decades. These factors include the total available organic matter, temperature, nutritional status of organic matter, plant community structure, availability of electron acceptors and water table levels (particularly the presence/absence of flooding) [e.g., Aerts and Ludwig, 1997; van Bodegom *et al.*, 2001; Keller *et al.*, 2004]. Ecosystems with high methane emissions include the boreal region, rice paddies and tropical wetlands.

[4] Global change might alter methane production in wetlands and emissions into the atmosphere directly and indirectly. The effects of increased temperature and water table height have been described extensively [e.g., Moore and Dalva, 1993; van Hulzen *et al.*, 1999; Price and Sowers, 2004], but also atmospheric N deposition, an important input to terrestrial ecosystems [Schlesinger, 2009], and elevated CO₂ may affect methane emissions. Direct effects of these factors result from changes in plant biomass. Both N deposition and elevated CO₂ may increase the amount of available soil organic matter due to their stimu-

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lation of plant and litter biomass production [Berendse et al., 2001; Heijmans et al., 2001; Reich et al., 2001]. Such increases in productivity are likely to increase CH₄ emission rates [Chidthaisong and Watanabe, 1997; Saarnio and Silvola, 1999]. Also CH₄ oxidation (mediated by methane-oxidizing bacteria) serves as an important control on CH₄ emission to the atmosphere and is inhibited at high NH₄ availability [Bodelier et al., 2000].

[5] Indirect effects result from changes during plant growth causing differences in the biochemical composition of plants [Norby et al., 2001; Tolvanen and Henry, 2001], which subsequently affect their decomposition rate in the soil and therefore CH₄ production. Increased CO₂ typically changes litter chemistry through an increased production of secondary carbon compounds [Poorter and Navas, 2003]. Although increased N deposition leads to a smaller tissue C/N ratios, subsequent decomposition rates may increase [Aerts and Chapin, 2000; Aerts et al., 2006], decrease [Magill and Aber, 1998; Gorissen and Cotrufo, 2000], or remain unaffected [Hobbie and Vitousek, 2000] depending on litter quality [Knorr et al., 2005]. Parallel to N deposition, an acceleration in N mineralization due to global warming is expected to further increase N availability [Mack et al., 2004]. To our knowledge there has been little research quantifying the effects of changes in litter quality on CH₄ emissions, even though such effects may potentially have global significance.

[6] The aim of this paper is to quantify the rate of methane production from litter of plants grown under the influence of increased atmospheric CO₂ and N availability. We expected elevated atmospheric CO₂ and increased N deposition to increase litter production, but that litter quality and hence methane production would be affected differently by these abiotic factors. We hypothesized that elevated CO₂ would lead to more secondary carbon compounds such as lignin and saturated fatty acids (lowering methane production rates), while increased N deposition would increase litter decomposability (raising methane production rates).

[7] We performed two complementary experiments: (1) a laboratory litter incubation experiment, where we studied litter mass loss, carbon mineralization and the contribution of CO₂ and CH₄ to these processes as affected only by changed litter quality under controlled, waterlogged conditions to test these hypotheses; and (2) a greenhouse pot experiment with the same litter used in experiment 1 added to waterlogged soil to validate the patterns in CH₄ emission.

2. Materials and Methods

2.1. Plant Material

[8] We used *Molinia caerulea* plants, a dominant species from wetlands throughout Northern and Western Europe, occurring in peatlands with water-saturated conditions in spring and dropping groundwater levels during summer. Methane emissions are known to occur in these systems [Lloyd et al., 1998]. To obtain ¹³C labeled litter of *M. caerulea*, intact PVC mesocosms of 25 cm diameter and 40 cm height were cut from a lowland peatland dominated by *M. caerulea* and *Sphagnum palustre*. The intact mesocosms were kept in a greenhouse in a factorial design with and without increased N deposition of 60 kg ha⁻¹ yr⁻¹ (N⁺ and N⁻, respectively), corresponding to twice the ambient

deposition in The Netherlands; and with and without elevated atmospheric CO₂ concentrations (of 700 and 400 ppm, C⁺ and C^a, respectively) in 6 replicates (N = 24). Although the mesocosms were kept in a greenhouse, the air temperature followed the natural annual course with minor differences. Nitrogen was applied as NH₄Cl. Other nutrients were applied in nonlimiting amounts: 0.8 g P/m², and 6.4 g K/m². During the third growing season, the mesocosms were spiked four times with ¹³C-enriched atmospheric CO₂ in all treatments and all the naturally senesced litter of this ¹³C-*M. caerulea* was subsequently collected to quantify total litter production rates. Six replicate air-dried and ground litter subsamples were analyzed for total organic carbon (C), total nitrogen (N) concentration and δ¹³C values. In addition, the abundance of major organic compounds was determined to evaluate litter quality (see below). This same litter was used in the laboratory litter-incubation experiment and in the greenhouse pot experiment.

2.2. Litter Incubation

[9] Rates of carbon mineralization and methane production were measured by incubating litter in glass jars. Glass jars (80 ml) were filled with 1.0 g of ¹³C-labeled *M. caerulea* litter cut into 5 cm long fragments. The litter was covered with 20 ml of water collected from remoistened fresh *M. caerulea* litter that had been soaked overnight in demi-water and filtered over a 100 μm Whatman filter, as litter-specific inoculum [Strickland et al., 2009] and to create waterlogged conditions.

[10] We used 6 replicates of litter per treatment combination. In addition, 6 jars without litter were prepared to control for CO₂ and CH₄ produced by the water alone. After closure, all jars were flushed with N₂ at 1 bar for 50 s to generate anoxic conditions [van Bodegom et al., 2005] and incubated them in the dark at a constant temperature of 20°C, optimal for litter decomposition [Aerts and de Caluwe, 1997]. We collected 100 μl headspace with a syringe at 3–4 days intervals to measure CH₄ and CO₂ concentrations and δ¹³C values. After each measurement we flushed again with N₂ and returned the jars to the 20°C incubator. After the tenth day of incubation, we added acetylene to 3 replicates of each treatment to quantify denitrification. Unfortunately, this also significantly decreased CH₄ production for the remainder of the incubation. Treatments with N fertilization did not recuperate from the very low values not even after gassing with N₂, suggesting that methanogens were completely inhibited in these treatments. These replicates were therefore excluded from further analysis. After 45 days the jars were opened and remaining litter was weighed after drying to a constant mass for 72 h at 70°C.

2.3. Pot Experiment

[11] Soil was collected from wet dune slacks in the western part of the Netherlands, an area known to be very poor in organic matter (soil total C = 0.66%, soil total N = 0.02%). The soil was sieved to minimize the contribution from sources of organic material other than the litter and thus maximize the sensitivity to differences in emissions induced by litter. Thirty pots of 0.011 m² and 20 cm high were filled with this soil and planted with *M. caerulea*. Each pot contained one plant with 4 to 7 tillers, and 13–35 leaves.

Neither initial number of leaves, nor number of tillers or leaf lengths was significantly different between treatments.

[12] All pots were placed in a greenhouse under controlled photoperiod (14 h light and 10 h dark), light intensity (250 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and temperature (17°C). In addition, we controlled the water level of the pots, mimicking flooded conditions with 1–2 cm standing water. The pots were equilibrated for 2 months during which time 50 ml of 5 mM acetate was added on a weekly basis to stimulate to stimulate methane production and electron acceptor consumption. Previous research under similar conditions has indicated that acetate additions decrease the redox potentials to values suitable for methane production, but do not negatively affect plant performance or general microbial activity [van Bodegom *et al.*, 2008].

[13] The ¹³C-labeled *M. caerulea* litter from each replicate of the original treatment combinations was cut into 1 cm long fragments. Litterbags (5 x 9 cm) with 1 mm² mesh size were filled with 0.35 g of this litter and two of them were randomly inserted just below the soil surface of each pot, after equilibration of the pots. In control pots, we simulated bag placement and subsequent root disturbance. This resulted in 4 treatments (given the 4 litter “types”) and 1 control, each replicated 6 times (N = 30).

[14] For gas sample collection, a ventilated chamber (60 cm high with a surface area of 0.011 m²) with water lock was temporarily installed on top of each pot [Matson and Goldstein, 2000]. Gas samples for CH₄ emission determination were collected by allowing gas exchange for 10 s through a double needle with a 5 ml gas vial that was previously evacuated. 5 ml samples, of which 500 μl was used for CH₄ analysis, were collected at 0, 5, 10, 15 and 20 min after positioning of the chamber. CH₄ production rates were calculated using regression assuming linear gas evolution (and rejecting calculated rates if $R^2 < 0.85$). At 20 min, an additional gas sample of 100 ml was collected for $\delta^{13}\text{C}$ analysis of accumulated CH₄. The ventilated chamber was removed immediately after sampling. Samples were collected at $t = 0, 7, 14, 21, 28, 35, 42$, and 49 days to quantify CH₄ emission dynamics from *M. caerulea* litter. No CO₂ was analyzed in this experiment, given the interference of plant photosynthesis and plant respiration on measured CO₂ signals. After 49 days, *M. caerulea* was harvested and the litter remaining in the litterbags was quantified.

2.4. Chemical Analysis

[15] Initial total organic carbon (C) and total nitrogen (N) concentration of the litter was measured on a Perkin Elmer 2400 series II CHNOS/O analyzer. $\delta^{13}\text{C}$ values of the litter were analyzed on a Carlo Erba EA1110 elemental analyzer coupled to a Finnigan Delta Plus IR-GCMS (isotope ratio–gas chromatograph/mass spectrometer).

[16] The relative abundance of major groups of macromolecular organic compounds, like saturated fatty acids, guaiacyl lignin, syringyl lignin, and p-hydroxyphenyl lignin, was determined through chemolysis of thermally assisted hydrolysis and methylation, as the number of detected ions in the mass spectrometer is linearly related to the amount of material [Blokke *et al.*, 2005]. Organic compounds were chosen to represent the components relevant for decomposition and were classified on the basis of their mass spectra, applying the commercial databases Wiley 6

and NIST98 [Yeloff *et al.*, 2008]. To 200 μg of sample, 5 μl of a 25% TMAH solution in methanol was added. The samples were incubated in a pyrolysis liner (CDS) at 70°C for 2 h and pyrolyzed at 550°C for 5 min in a CDS AS-2500 pyrolysis unit (CDS Analytical Inc.) (260°C interface temperature) coupled to an Agilent 6890 GC equipped with an Agilent 5973 MSD. The GC oven was programmed from 40°C (5 min hold time) to 130°C at 20°C/min and subsequently to 320°C at 6°C/min followed by 10 min isothermal at 320°C. A HP5-MS capillary GC column was used with helium as carrier gas at a constant flow of 1.2 ml/min in a 20:1 split ratio. The mass spectrometer was operated in full-scan mode (m/z 50–800) at 70 eV ionization energy [Blokke *et al.*, 2005].

[17] We determined total CH₄, N₂O and CO₂ concentrations on a Hewlett Packard 5890A gas chromatograph equipped with a 25 m CarboPlot P7 column and a flame ionization detector for CH₄ and a thermal conductivity detector for N₂O and CO₂. The minimum flux detection limit was approximately 0.5 ppm CH₄. The $\delta^{13}\text{C}$ values of CH₄ were determined with a Finnigan PreCon/Gasbench coupled to a Finnigan Delta Plus IR-GCMS. For this purpose, we used an analysis routine that first removes all CO₂, second oxidizes all CH₄ to CO₂ and then measures $\delta^{13}\text{C}$ values at an accuracy of 0.1 ‰. The volume injected into the GCMS was estimated using the CH₄ concentration detected by GC.

2.5. Calculations

[18] In both experiments, measured CH₄ may have been produced from litter or from other sources of organic matter, such as organic matter dissolved in the water or in the soil of the pot experiment. To isolate the effects of litter on CH₄ production, a ¹³C mass balance was used:

$$\text{AT}\%^{13}\text{C}_{\text{measured}}/100 \cdot \text{CH}_{4\text{measured}} = \text{AT}\%^{13}\text{C}_{\text{o.m.}}/100 \cdot \text{CH}_{4\text{o.m.}} + \text{AT}\%^{13}\text{C}_{\text{litter}}/100 \cdot \text{CH}_{4\text{litter}}$$

where CH₄ is CH₄ release, and AT%¹³C is the atom percentage of ¹³C over total C measured in released CH₄ (to account for fractionation of ¹³C during conversion of organic C to CH₄). For AT%¹³C_{litter}, we considered the value corresponding to the last measurement of ¹³C for CH₄ produced from litter at the laboratory litter incubation, assuming that litter was the only source of CH₄ remaining during the last period of incubation. This value was used both for the litter incubation and pot experiments. CH₄ production and AT%¹³C from sources of available organic matter other than litter was obtained from controls without litter.

2.6. Statistical Analysis

2.6.1. Litter Quality and Mass Loss

[19] The effects of N fertilization and of CO₂ concentration during plant growth on initial litter C, initial litter N and total litter mass loss in both experiments were analyzed using a two-way ANOVA. Weights of the duplicate litter bags within each pot were averaged. Effects of control versus treatments were tested with one-way repeated measures ANOVA (rmANOVA) with time as within-subjects factor and a Dunnett post hoc test at each time.

Table 1. Litter Parameters From Original Mesocosms^a

	C ⁺ N ⁻	C ⁺ N ⁺	C ^a N ⁻	C ^a N ⁺	N Fertilization	CO ₂	N*CO ₂
Litter production (gm ⁻² yr ⁻¹)	236.5 (17.7)	202.3 (24.7)	186.6 (17)	190.6 (27.4)	NS	NS	NS
Litter C (%)	44.93 (0.11)	45.64 (0.09)	44.93 (0.09)	45.58 (0.10)	**	NS	NS
Litter N (%)	0.54 (0.003)	0.94 (0.005)	0.60 (0.004)	0.96 (0.003)	**	**	**
C/N ratio	83.7 (0.39)	48.3 (0.31)	74.2 (0.58)	47.6 (0.15)	**	**	**
δ ¹³ C values (‰)	315 (3.45)	702 (3.45)	293 (1.31)	716 (4.50)	**	NS	**

^aMean (±SE, given in parentheses, N = 6) litter production from original mesocosms, initial carbon and nitrogen concentration, C/N ratio, δ¹³C value per dry mass of *M. caerulea* litter from plants grown under the combination of two treatments: CO₂: 400 and 700 ppm CO₂ (C^a and C⁺, respectively) and N fertilization: with and without increased 60 kg ha⁻¹ yr⁻¹ N (N⁺ and N⁻, respectively). **P < 0.01.

[20] Patterns in composition of major groups of macromolecular organic compounds in the litter were determined by redundancy analysis (RDA), constrained and explained by the N fertilization and CO₂ treatments (direct gradient analysis). A RDA was chosen, because a detrended correspondence data analysis showed that the gradient length of the first axis was around one [Leps and Šmilauer, 2003]. Scaling was focused on interspecies correlations, species data (i.e., organic compounds) were centered and species scores were not posttransformed. The significance of the RDA axes was determined in a Monte Carlo test with 499 permutations. All ordination analyses were performed using Canoco 4.5 [ter Braak and Šmilauer, 2002].

2.6.2. Carbon Mineralization and CH₄ Production and Emission Rates

[21] The effects of N fertilization and of CO₂ concentration during plant growth on carbon mineralization rates, CH₄ production/emission rates, CH₄ produced/emitted from litter, and δ¹³C values were analyzed by two-way rmANOVA with the treatments as fixed factors and time as within-subjects factor for both the litter incubation and the pot experiment. Carbon mineralization data and CH₄ production and emission data were log-transformed prior to testing to attain normality and homogeneity of residuals. Inspection of the residuals in plots of normalized residuals versus predicted values confirmed that the homogeneity of variances was satisfactory and that residuals approached a normal distribution after this transformation.

3. Results

3.1. Litter Quality

[22] Litter production of *M. caerulea* did not differ significantly among the treatments in the intact mesocosms from the lowland peatland (Table 1). Litter of plants that had received N fertilization (N⁺) had higher C and N concentrations and a lower C/N ratio than litter receiving low N (N⁻) (Table 1). Also, plants that had grown under elevated CO₂ had a lower N concentration than litter from ambient CO₂. The concentration of N was further affected by the interaction among the treatments: under N⁻, the concentration of N was 11% lower for elevated CO₂ in comparison to ambient CO₂, while the N concentration was only 1.5% lower under N⁺. The C/N ratios followed the same pattern as the N concentrations, in being affected by N fertilization, CO₂ concentration and their interaction. Initial litter had

been enriched by ¹³C and was, coincidentally, higher with N fertilization than without N fertilization due to random differences in length of the labeling period and C uptake during that period (Table 1).

[23] The composition of organic macromolecular compounds in the litter was also strongly affected by N fertilization and CO₂ concentration (Figure 1): The first canonical-RDA axis explained 28% of the total variability in the organic compounds, while an additional 18% was explained by the second RDA axis. Both axes were constrained by the treatments and were significant (P < 0.05 and P < 0.01, respectively). So, the imposed treatments led to strong changes in the composition of pyrolyzed organic compounds, explaining in total 46% of the variation.

[24] The treatments primarily led to changes within the guaiacyl-lignin fragments and wax compounds. In the majority of cases, the treatments led to the replacement of one guaiacyl-lignin fragment or wax compound by the other (Figure 1). Elevated CO₂ on average led to an increase in guaiacyl-lignin fragments, whereas N fertilization on average led to lower abundance of wax compounds. Results were very similar when a RDA using the individual treatments as dummy environmental factors was performed (data not shown).

3.2. Litter Incubation

[25] Based on the lower C/N ratios (Table 1) and lower abundance of wax compounds (Figure 1), increased decomposability upon N fertilization was expected, while the higher C/N ratios and higher guaiacyl-lignin abundance under elevated CO₂ would indicate lower decomposability. However, no large differences in mass loss from *M. caerulea* litter were observed. After 48 days, approximately 20% of the original litter had been decomposed. Mass loss from litter under ambient CO₂ was about 2% higher than from litter under elevated CO₂ (F = 5.23, P < 0.05, Figure 2a), but there were no N fertilization effects. Treatment effects on cumulative C mineralization over the same time period were even smaller: Cumulative C mineralization was not affected by any of the treatments (Figure 3a). C mineralization rates were higher at the start of the incubation and decreased with time (F = 130.03, P < 0.07), stabilized to linear accumulation rates from the third week onward (Figure 3a). Cumulative C mineralization from controls was significantly lower than from any treatment during the whole experiment (F = 19.96, P < 0.05; Dunnett Test P <

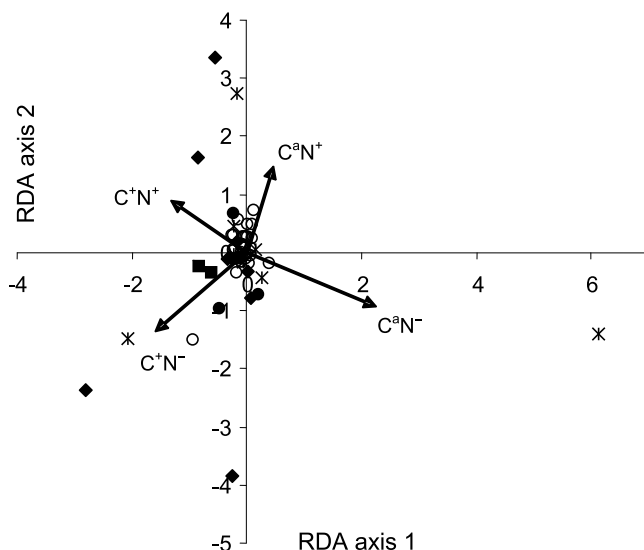


Figure 1. Biplot from a redundancy analysis (RDA) with treatments (as environmental variables, arrows) and organic compounds in the litter (species; saturated fatty acids, asterisks; guaiacyl lignin, diamonds; syringyl lignin, squares; p-hydroxyphenyl lignin, circles) determined through chemolysis-MS. Axis 1 (horizontal) explains 28% of the total variance, and axis 2 (vertical) explains 18%. Litter was collected from plants grown at 400 and 700 ppm CO₂ (C^a and C⁺, respectively) and with or without increased 60 kg ha⁻¹ yr⁻¹ N (N⁺ and N⁻, respectively).

0.05 at each point in time). The same was true for cumulative CO₂ production (data not shown), which was an order of magnitude higher than CH₄ production, and which dominated total C mineralization.

[26] Patterns in cumulative total CH₄ production (Figure 3b) and the calculated cumulative CH₄ produced from the litter (Figure 3c) were similar, reflecting that CH₄ production rates in the controls were constant in time. Only the treatments without N fertilization produced more CH₄ than the controls (Dunnett Test $P < 0.05$ for the fourth week onward). The cumulative CH₄ production from litter was lower with N fertilization ($F = 43.17$, $P < 0.01$; Figure 3c), but was unaffected by CO₂ concentration. However, the N fertilization effect varied with CO₂ concentration: Under N⁻, cumulative CH₄ production was higher under ambient CO₂ than under elevated CO₂, while with N⁺, CH₄ production was higher under elevated CO₂ than under ambient CO₂. Cumulative CH₄ production increased with time ($F = 166.1$, $P < 0.01$).

[27] The $\delta^{13}\text{C}$ -CH₄ signature of the treatments was enriched compared to controls (Dunnett test, $P < 0.05$), that had $\delta^{13}\text{C}$ values of -55 to -50 ‰ (Figure 4) throughout the experiment. The $\delta^{13}\text{C}$ of CH₄ emitted by each treatment approached that of each treatments respective litter $\delta^{13}\text{C}$ values. $\delta^{13}\text{C}$ -CH₄ attained the litter $\delta^{13}\text{C}$ values in both treatments without fertilization (N⁻) after 20 days. Thus, the CH₄ produced was mainly derived from *M. caerulea* litter. $\delta^{13}\text{C}$ -CH₄ in the treatment with ambient CO₂ and fertilization (C^aN⁺) was similar to that of the initial litter at the start of the incubation, but decreased afterward, while $\delta^{13}\text{C}$ -CH₄ of the elevated CO₂ and fertilization treatment (C⁺N⁺)

always remained lower than the initial value of the corresponding litter. The lower $\delta^{13}\text{C}$ signal of C⁺N⁺ suggests an extra source of CH₄ from an alternative organic matter source to litter; this assertion is compatible with the lower decomposition in this treatment.

3.3. Pot Experiment

[28] Litter decomposition of *M. caerulea* was not affected by the treatments (Figure 2b). After 45 days, approximately 20% from the original litter had decomposed.

[29] Cumulative CH₄ emissions from the pots were highly variable within treatments, and no treatment effect was detected (Figure 5a). CH₄ produced from litter contributed less than 25% to the total CH₄ emissions and was not affected by treatments (Figure 5b), indicating that soil organic matter served as main source of CH₄. The high within-treatment variability was unrelated to plant biomass or number of tillers (which have been used as covariables to decrease within-treatment variability in the past [Denier van der Gon and Neue, 1996]). Cumulative CH₄ emissions increased until the third and fourth weeks, for C^a treatments and C⁺ treatments, respectively (Figures 5a and 5b). Thereafter, CH₄ produced from litter leveled off for all treatments (Figure 5b, time effect $F = 181.4$ and $F = 14.0$, $P < 0.01$ for total CH₄ emission and CH₄ emission from litter, respectively).

[30] The temporal dynamics of $\delta^{13}\text{C}$ -CH₄ values were synchronous to the CH₄ emissions (Figure 6), but remained much lower than the original $\delta^{13}\text{C}$ values of the litter. The $\delta^{13}\text{C}$ values of emitted CH₄ were significantly higher for treatments with litter grown under N fertilization, compared to those without N fertilization, reflecting the coincidental initial differences in $\delta^{13}\text{C}$ litter labeling ($F = 12.28$, $P < 0.05$). However, this effect varied marginally with time ($F =$

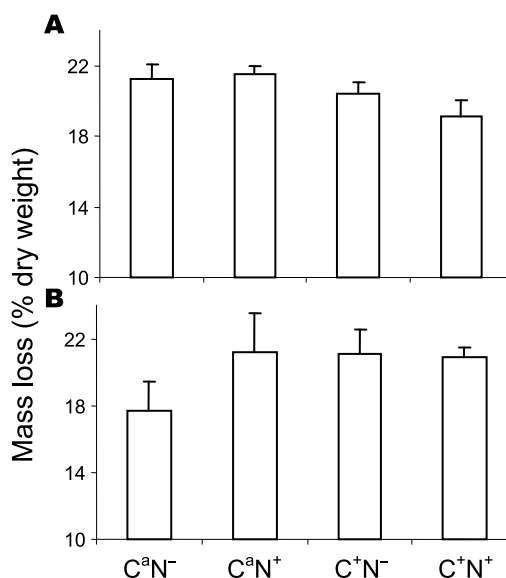


Figure 2. Mass loss of *M. caerulea* litter from plants in (a) a laboratory experiment incubating litter for 48 days ($N = 24$) and (b) a greenhouse pot experiment with mass loss measured over 49 days ($N = 24$). Treatments are as explained for Figure 1, and each bar represents the mean and standard error of the mean.

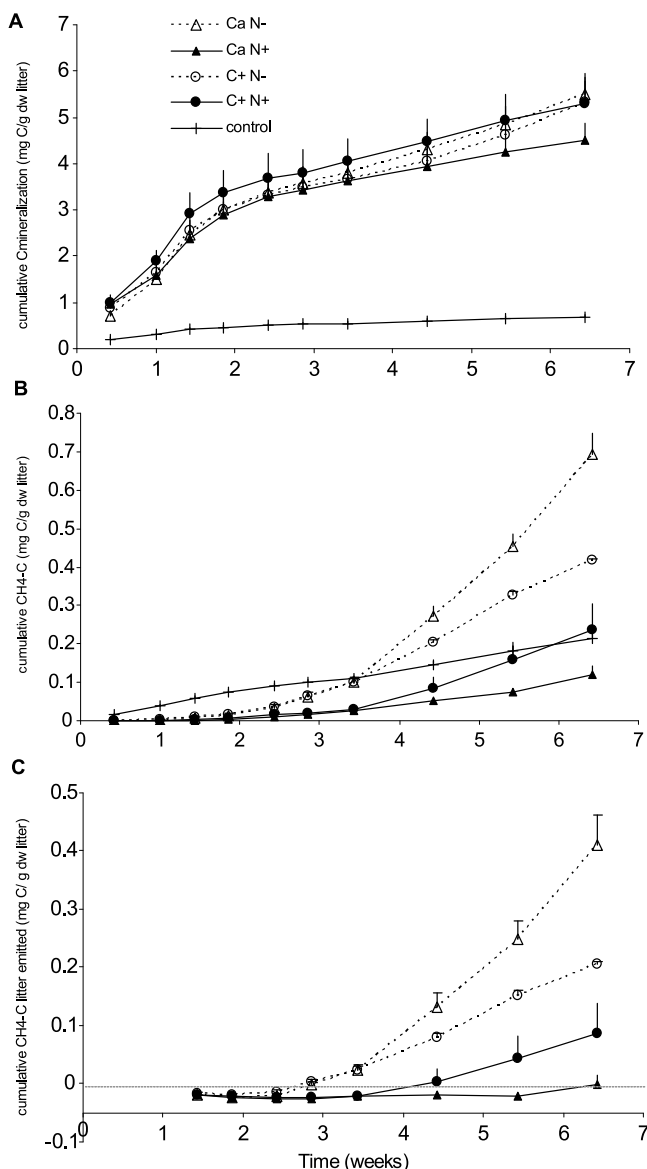


Figure 3. Cumulative carbon dynamics with (a) C mineralization (CO₂ + CH₄), (b) cumulative total CH₄ production, and (c) production of CH₄ from litter (correcting for CH₄ release from controls) from jars with litter of *M. caerulea* in a laboratory incubation. Values are expressed in grams of carbon of CH₄ and/or CO₂ per gas volume in the jars. Negative values in Figure 3c indicate that CH₄ emission from controls (to calculate CH₄ emitted from other organic matter sources) was higher than that in treatments. N = 3 per treatment. Bars represent standard error of the mean. Classification of treatments follows Figure 1.

2.73, $P = 0.054$, for N fertilization*time interaction) as the differences between N⁺ and N⁻ decreased as the experiment progressed, suggesting that CH₄ production from litter grown in the absence of N fertilization was higher. The CO₂ concentration effect on $\delta^{13}\text{C}$ -CH₄ values also varied with time. At the end of the experiment, elevated CO₂ treatments had higher $\delta^{13}\text{C}$ -CH₄ values than those from the ambient CO₂ treatments ($F = 5.18$, $P < 0.01$, for CO₂ con-

centration*time interaction), whereas initial differences in $\delta^{13}\text{C}$ -CH₄ values were not significant.

4. Discussion and Conclusions

4.1. Litter Quality

[31] The reported decreases in N concentrations under elevated CO₂ and the consequent increases in C/N ratios [Cotrufo and Ineson, 1996; Berntson and Bazzaz, 1998] have been linked to a larger accumulation of carbon-based secondary compounds, like lignins, polyphenols and waxes in plant tissues [Peñuelas et al., 1996; Peñuelas and Estiarte, 1998; Hu et al., 1999]. Indeed, using chemolysis and RDA analysis, we confirmed that elevated CO₂ increased the relative abundance of lignin. Low N availability is associated with increased concentrations of carbon-based secondary compounds [Herms and Mattson, 1992; Hättenschwiler and Vitousek, 2000] and was associated with increased abundance of wax compounds in our study. Even so, the chemolysis analysis, in which the majority of the macromolecular organic compounds were identified, showed that shifts in composition of these compounds were more important than absolute increases in their abundance.

[32] The absolute effects of elevated CO₂ were small compared to those of N fertilization, also reported in several reviews [Hirschel et al., 1997; Norby et al., 2001; Finzi and Schlesinger, 2002]. Overall, the effects of our treatments on litter quality were similar to those typically reported in the literature, although the details of changes were more precisely quantified in our study. This makes our study appropriate to separate the effects of global change factors mediated through litter quality versus litter production on CH₄ production. Unfortunately, to explicitly separate these two factors, a common soil environment had to be used to avoid differences in litter quantity, but as in our study, the use of common soil limits the discrimination of potential changes to the soil microbial community due to elevated CO₂/N deposition (although the effects of potential changes

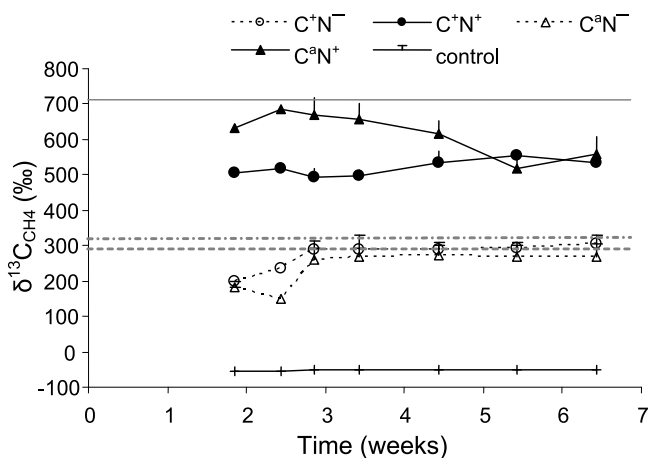


Figure 4. The $\delta^{13}\text{C}$ values of methane emitted from litter of *M. caerulea* in a laboratory incubation. The lines represent the respective initial $\delta^{13}\text{C}$ values of litter: C^aN⁺ and C⁺N⁺ (solid lines); C^aN⁻ (dashed lines), and C⁺N⁻ (dot-dashed lines).

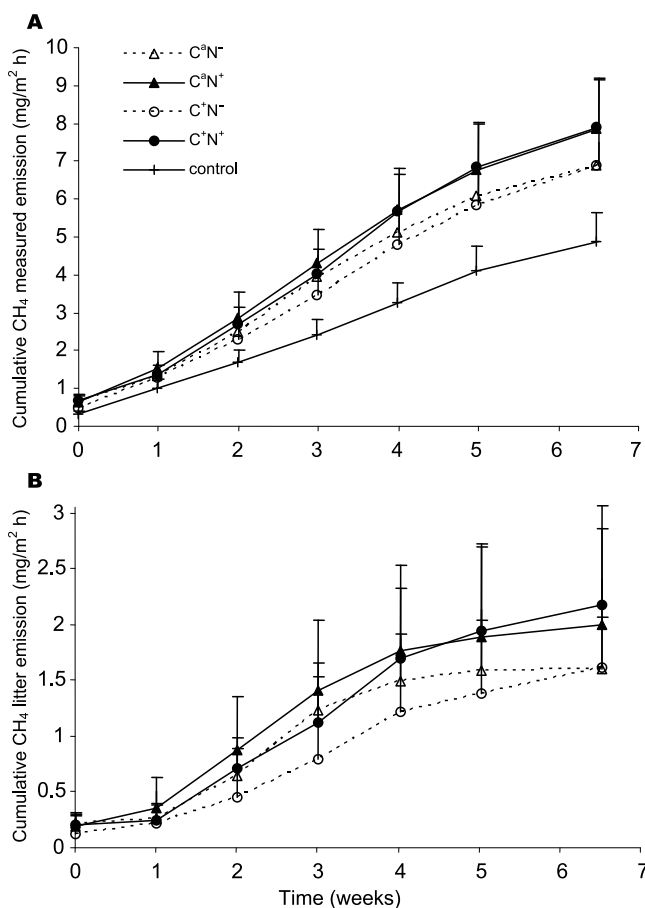


Figure 5. CH₄ emissions from pots planted with *M. caerulea* and containing *M. caerulea* litter in a pot experiment. (a) CH₄ emissions measured from pots and (b) calculated CH₄ emission from litter. Classification of treatments follows Figure 1.

in soil microbial community may be marginal) [Allison and Martiny, 2008].

4.2. CH₄ Production Responds Differently Compared to Total Carbon Mineralization

[33] Methane production measured in the litter incubation under anoxic conditions followed the expected temporal dynamics, increasing with time following the expected depletion of the pools of alternative electron acceptors from water and *M. caerulea* litter. At the end of the incubation CH₄ predominantly came from litter, as reflected in the $\delta^{13}\text{C}$ values that approached the $\delta^{13}\text{C}$ values of the litter itself. The convergence of $\delta^{13}\text{C}$ values of produced CH₄ with those of the labeled litter, in the laboratory litter incubation, provides circumstantial evidence that the litter from different treatments had been labeled uniformly with ¹³C.

[34] In the short-term laboratory incubation experiment, the treatments affected CH₄ production differently compared to C mineralization, which is an integrative measure of the available carbon substrates for CH₄ production. While CH₄ production decreased upon N fertilization and was unaffected by CO₂ elevation, there was no effect of N fertilization upon C mineralization and litter mass loss, but decreased mass losses of *M. caerulea* litter at elevated CO₂

(the latter coinciding with decreased litter quality [Gorissen *et al.*, 1995; Hirschel *et al.*, 1997]). Thus, differences in the availability of precursors for methane production (like acetate and H₂/CO₂) did not explain the effects of the global change factors on CH₄ production. This also implies that differences in lignin and other secondary carbon compounds, or differences in litter decomposability in general, do not explain the patterns in CH₄ production rates in our study.

[35] In our study, decreased CH₄ production from fertilized litter was not due to increased denitrification either, which would have been possible due to higher concentrations of litter N, as a secondary compound that acts as alternative electron acceptor [Knowles, 1979]. Using the acetylene inhibitor method, we did not detect N₂O in any gas samples, indicating that no detectable denitrification had occurred (data not shown). Low denitrification rates below the detection limit of the system would not have affected CH₄ production rates so strongly. If denitrification had occurred during the first days of incubation (acetylene was added after 10 days), then the inhibiting effects of denitrification should have been extinguished afterward. This did not happen, thus we conclude that differences in denitrification do not explain the treatment effects of N fertilization on methanogenesis either.

[36] Alternatively, it has been reported that methanogens may be sensitive to ammonium and gaseous N-containing products [Hendriksen and Ahring, 1991; Klüber and Conrad, 1998; van Bodegom and Scholten, 2001]. Although it is still difficult to determine the mechanism of toxicity and which type of methanogens are affected [Sawayama *et al.*, 2004], this may explain why the pathways of total carbon mineralization and CH₄ production were affected differently by N fertilization.

[37] In the pot experiment, effluxes of CH₄ were similar to those in the laboratory incubation, but the variability in effluxes within treatments was much higher, obscuring any significant treatment effect. This variability that occurred

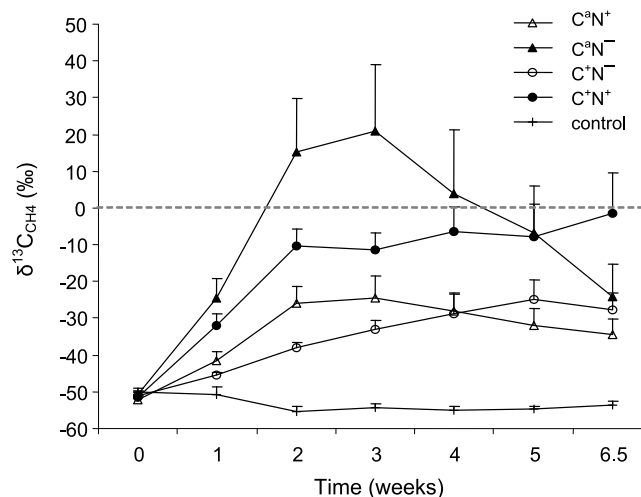


Figure 6. The $\delta^{13}\text{C}$ values of CH₄ emitted from pots planted with *M. caerulea* and containing *M. caerulea* litter in a pot experiment. Classification of treatments follows Figure 1.

under less controlled conditions may reflect that in semi-natural conditions other factors or mechanisms are involved, that we did not control in the pot experiment, and that also affect CH₄ emissions. In our pot experiment, leaf litter was inserted in the water-saturated topsoil layer in order to maximize the contribution of leaf litter to methane, compared to other carbon sources like root materials. This setup should thus maximize the capacity to detect potential indirect effects through litter quality on methane production. Still, treatment effects were insignificant and the $\delta^{13}\text{C}$ labeling of the pot experiment produced much lower values than the laboratory litter incubation, reflecting that litter was a minor source of CH₄ in the pot experiment (compare Figures 5a and 5b [Chidthaisong and Watanabe, 1997; Watanabe et al., 1998; von Fischer and Hedin, 2007]). Note that partial oxidation of methane would increase $\delta^{13}\text{C}$ of emitted methane, overestimating the role of labeled leaf litter to methane emissions and even further decreasing the actual role of this carbon source. The relative contribution of CH₄ from litter was fairly constant, as reflected in the low variation in $\delta^{13}\text{C}$. The natural variability in the other sources of CH₄ thus probably caused the nonsignificant treatment effects in CH₄ emissions. The relative increase in $\delta^{13}\text{C}$ for N⁻ treatments compared to the N⁺ treatments shows, however, that CH₄ production from unfertilized litter gained importance during the experiment, consistent with the laboratory incubation.

4.3. Potential Consequences of the Differential Response of CH₄ Production

[38] Studies on the effects of climate change on soil carbon respiration [Mack et al., 2004] and litter decomposition [Knorr et al., 2005; Aerts et al., 2006] in peat ecosystems have consistently reported an increase in carbon fluxes under N deposition and elevated CO₂. These effects on litter decomposability have been attributed partly to influences of increased N availability [Mack et al., 2004] as N mineralization rates also rise [Rustad et al., 2001]. Although it is appealing to assume that CH₄ production rates will respond similarly to these indirect effects of global change, as they comprise part of the soil carbon cycle, we found that CH₄ production responds in a different way, as the factors controlling its production seem to be different to those for total carbon respiration.

[39] In contrast to the anticipated increase in concert with other carbon fluxes, and in contrast to potential amplification of responses for methane due to inhibiting effects of N compounds on CH₄ oxidation [Kravchenko, 2002], CH₄ production rates from litter decreased following N fertilization of *M. caerulea* plants. These decreases were large and ranged from 50% (for litter produced at elevated CO₂ concentrations) to 80% (for litter from ambient CO₂ concentrations). These indirect effects of N availability on CH₄ production, on which hitherto no data were available, also contrast with the combined effects of N fertilization through litter production and litter chemistry on CH₄ production rates. Combined effects for peatlands show only a transitory stimulation of CH₄ efflux [Aerts and Toet, 1997; Saarnio and Silvola, 1999; Nykänen et al., 2002] or no significant response [Dise and Verry, 2001]. This small stimulation is somewhat unexpected in the light of the generally increased

litter biomass production upon N fertilization [Berendse et al., 2001; Heijmans et al., 2001; Reich et al., 2001], although there were no significant differences in litter production under the different treatments in our study.

[40] The strong negative effect of N deposition on CH₄ production through litter N availability in this study may explain, however, why the combined effects of N deposition are only modest. This implies that it is important to account for both the direct and indirect effects when predicting CH₄ emissions from wetlands, although presently none of these effects are accounted for in any global CH₄-emission model on wetlands. This may particularly affect predictions for regions with high N deposition [Dentener et al., 2006] and high CH₄ emissions from wetlands [e.g., Shindell et al., 2004], like central Europe and northern China.

[41] Overall, our study shows that the indirect effect of N deposition through changes in litter N availability may potentially depress CH₄ production, and that these effects interact with those induced by differences in atmospheric CO₂ concentrations. More research over various conditions is needed to test the generality of these indirect effects in comparison to direct effects of N availability on CH₄ production.

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